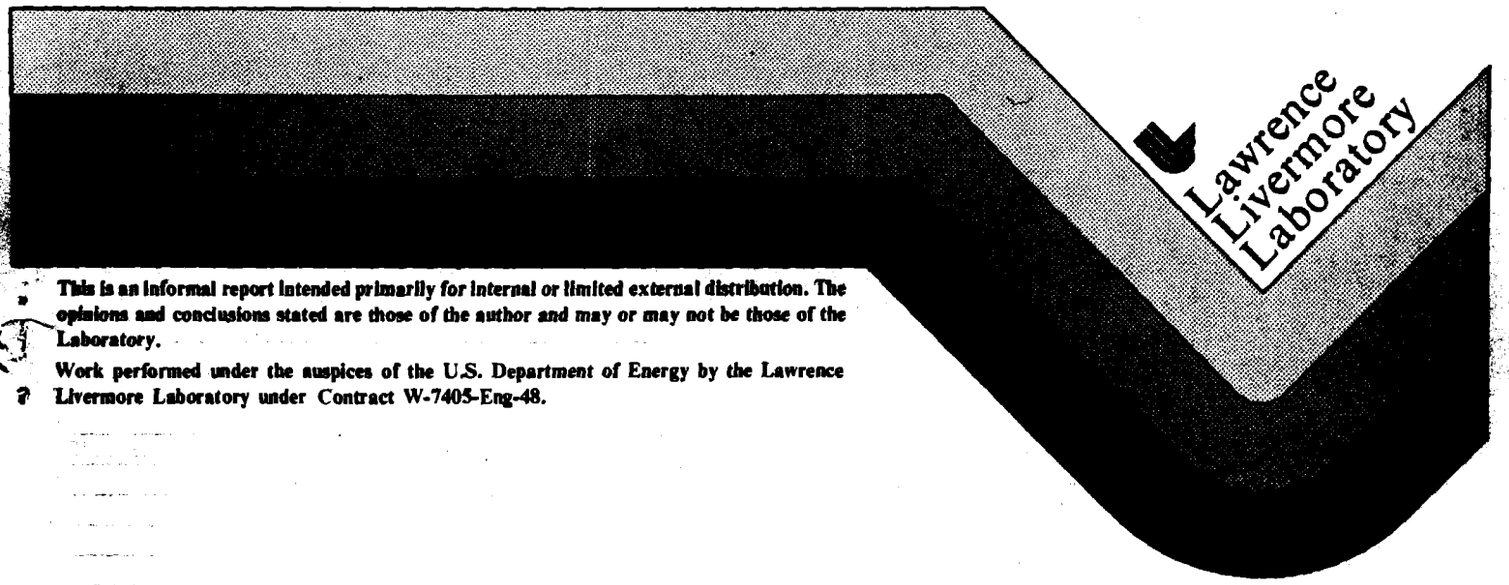
UCID- 19993

POLLUTANT BODY BURDENS AND REPRODUCTION IN *PLATICHTHYS STELLATUS* FROM SAN FRANCISCO BAY

Robert Spies
David Rice
Robert Ireland
John Beach

August 1983



POLLUTANT BODY BURDENS AND REPRODUCTION IN *PLATICHTHYS STELLATUS* FROM SAN FRANCISCO BAY

ANNUAL PROGRESS REPORT

YEAR I

Robert Spies

David Rice

Robert Ireland

John Beach

Environmental Sciences Division

University of California

Lawrence Livermore National Laboratory

Livermore, California 94550

Prepared for

Dr. Edward Long
National Oceanic and Atmospheric Administration
National Ocean Service
Office of Oceanography and Marine Services
Ocean Assessments Division
Coastal and Estuarine Assessment Branch
Seattle Project Office
7600 Sand Point Way N.E., BIN C15700
Seattle, Washington 98115

August 1983

INTRODUCTION

This progress report summarizes our efforts and results during the first year of the grant. It includes information reported in the first semi-annual progress report (April, 1983). In some cases the previously-reported results have been superseded by the inclusion of more data and further statistical analysis. This report gives a more complete account of organ-body indices, age structure of populations and the relationships of mixed-function oxidase activity to various biological parameters. A major new effort has been the chemical analyses for pollutants in a series of oocytes with varying degrees of fertilization success.

To restate briefly the goals of the project, we are determining if sublethal levels of pollutants in the tissues of an estuarine fish are affecting reproductive success. Our approach has been to examine the relationships among several measures of reproductive success (percent fertilization, hatching success, abnormal larvae) and biochemical measures of pollutant exposure (induced mixed-function oxidase) as well as direct measures of chemical pollutants in tissues. Our approach has been to sample the populations during active gametogenesis (October, 1982) and then again during the spawning season (December, 1982 - February, 1983). For all the collections, we have determined various body indices, including gonad-to-body (gonadosomatic) and liver-to-body (hepatosomatic) indices and otolith age estimates. Tissues have been preserved for pollutant analyses. During the December, 1982, through February, 1983, collections we brought fish back to the laboratory for spawning. We evaluated the reproductive success of spawning females by the above mentioned criteria. Hepatic mixed-function oxidase (MFO) activity has been measured in a large number of captured fish.

We believe that results obtained so far in the study indicate that the reproductive success of starry flounder in San Francisco Bay is being impaired by organic pollutants, particularly in the mid-bay near Berkeley. There are several lines of evidence that indicate that reproductive effects are correlated with high activity levels of pollutant-induced forms of cytochrome P-450-linked MFO.

The major trends in our data indicate that

(1) San Francisco Bay fish were significantly smaller than Monterey Bay fish, based on analysis of their growth curves.

(2) Within San Francisco Bay, year-1 fish collected from Berkeley were significantly smaller than those collected from San Pablo Bay.

(3) Fish collected near Berkeley, within San Francisco Bay, have the highest mean MFO specific activity and the lowest percentages of fertilization success.

(4) Fish collected from the less urbanized San Pablo Bay, within San Francisco Bay, have low MFO specific activities and (a) the highest mean percentage of fertilization success, (b) the lowest hatching success of any of the spawned populations, and (c) the greatest mean percentage of abnormal larvae.

(5) In environments such as Berkeley, which is heavily urbanized and polluted with MFO inducers, total hepatic MFO activity is significantly related to body size and enlarged livers.

(6) Higher hepatic MFO specific activities are significantly correlated with lower fertilization success.

(7) A series of chlorinated hydrocarbons, mainly DDT-type compounds and polychlorinated biphenyls (PCBs), have been identified in the oocytes of spawning females. Chlorinated hydrocarbons tend to be in higher

concentrations in fish from Berkeley. Relationships of reproductive success and contaminants, however, are tentative and require further data in the second year of the study.

FIELD SAMPLING

A total of 74 fish were collected in the Monterey and San Francisco Bays during October-November, 1982. These fish came from the Moss Landing-Pajaro River area of central nearshore Monterey Bay, from San Pablo Bay and from central San Francisco Bay (Fig. 1, Table 1a). During this period we relied on the California Department of Fish and Game, which conducts a one-week sampling effort throughout San Francisco Bay every month. Twenty-nine of the 74 fish came from Fish and Game trawls. Their sampling program in October and in earlier months indicated that the Central Bay area, especially near Berkeley and Richmond, and San Pablo Bay had the largest populations of starry flounder and that the South Bay (below the Alameda-South San Francisco line) and the areas beyond the Carquinez Straits (Suisun Bay and the Sacramento-San Joaquin River Delta system) would not produce enough adult fish, if any, to justify a large sampling effort there later in the spawning season. An additional twenty starry flounders came from a shrimp fisherman in a single balloon trawl NW of Vallejo in San Pablo Bay. An intense effort was needed in Monterey Bay to capture 21 flounders. These fish were captured at a rate of only 1 or 2 fish per 20 min otter trawl sample.

A total of 182 starry flounders were collected between December 6, 1982, and February 23, 1983. The collecting effort and the disposition of the fish are summarized in Tables 1a and 1b. We had originally hoped to capture running ripe fish in the field, but none of the female fish produced ripe gametes at the time of collection. We tried to get gametes by gently massaging the

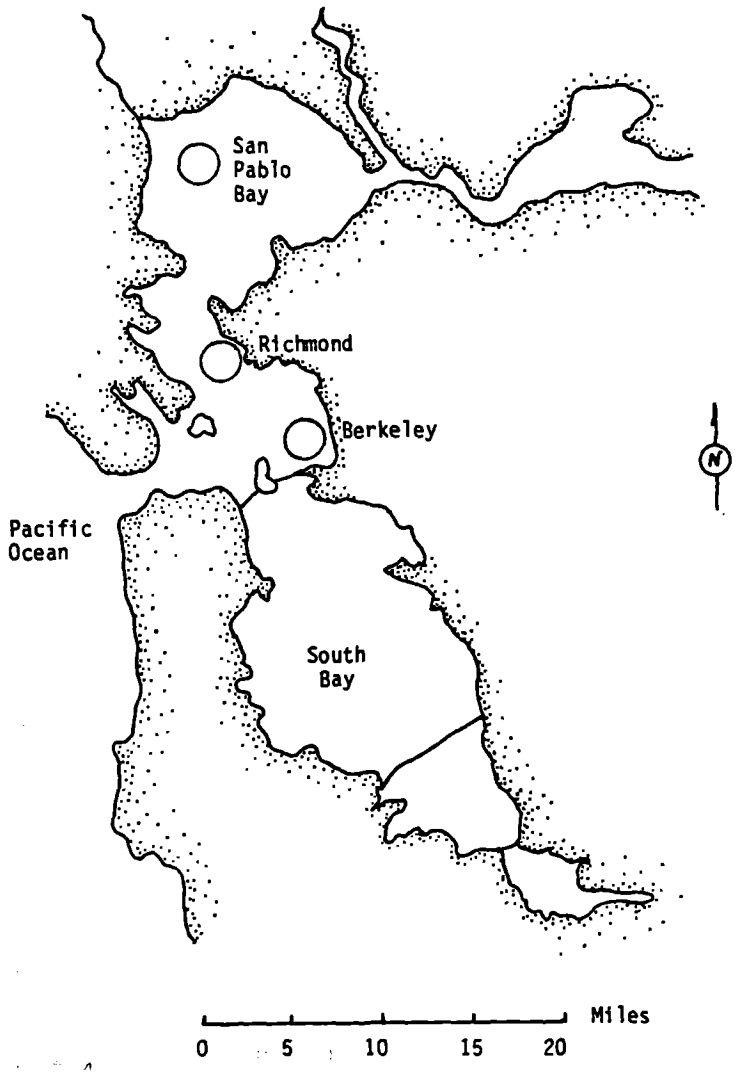


Figure 1. San Francisco Bay with major starry flounder collection locations indicated.

abdominal area of each fish, but we were unsuccessful. Our experience was the same as that of Dr. Policansky, Gray Herbarium, Harvard University (personal communication) who had unsuccessfully trawled for ready-to-spawn fish in San Francisco Bay. Two days of intensive trawling near the mouth of the Pajaro

Table 1a. Sampling effort in 1982.

Date	Location	Fish collected (Accession number)	Disposition
		October	
10/5-10/7/82	Moss Landing area[a]	22 (MBS 2342-2363)	All fish dissected
10/12/82	Upper San Pablo Bay[b]	15 (MBS 2364-2378)	"
10/13/82	Berkeley area[c]	14 (MSB 2379-2390)	"
10/19-10/20/82	Upper San Pablo Bay[d]	22 (MBS 2391-2412)	"
		November	
11/10/82	Moss Landing area[a]	1 (MSB 2413)	Injected with carp pituitary CP
		December	
12/6/82	Upper San Pablo Bay[b]	38 (MSB 2414-2451)	All fish dissected (immature)
12/14-12/15/82	Berkeley[e]	48 (MSB 2452-2455 MSB 2457-2468 MSB 2499X-2503X MSB 2504-2530)	MSB 2457-2468 injected with CP, (rest dissected for otoliths only)
	Richmond[f]	10 (MSB 2456) (MSB 2469-2477)	MSB 2469-2477 injected with CP
	San Pablo Bay[g]	32 (MSB 2478-2489 MSB 2574-2594)	MSB 2478-2489 injected, (rest dissected for otoliths only
12/20-12/21/82	Moss Landing area[a]	4 (MSB 2495-2498)	all injected with CP

Table 1b. Sampling effort in 1983.

Date	Location	Fish collected (Accession number)	Disposition
		January	
1/7/83	Berkeley[e]	10 (MSB 2531-2540)	all injected with CP
	Richmond[f]	2 (MSB 2541-2542)	all injected with CP
	San Pablo Bay[g]	8 (MSB 2543-2550)	all injected with CP
1/17/83	Berkeley[e]	15 (MSB 2551-2565)	injected with CP MSB 2551-2556 (rest dissected)
	Richmond[f]	6 (MSB 2567-2573)	injected with CP MSB 2567-2571 (rest dissected)
		February	
2/3/83	Berkeley[e]	2 (MSB 2603-2604)	all injected with CP
	San Pablo Bay[g]	8 (MSB 2595-2602)	all injected with CP
2/10/83	Moss Landing[a]	5 (MSB 2607-2611)	injected with CP MSB 2607-2608 (rest dissected)
2/23/83	Berkeley[e]	3 (MSB 2612-2614)	all injected with CP

[a] One-half mile north and south of the mouth of the Pajaro River.
[b] Fish collected by California Department of Fish and Game from stations in the upper San Pablo Bay and Carquinez Straits.
[c] Fish collected by California Department of Fish and Game from stations in mid-San Francisco Bay.
[d] Fish collected in upper San Pablo Bay and Carquinez Straits by shrimp boat working out of Crockett Marina.
[e] Fish collected in approximately 10 feet of water, south of Berkeley Pier to north of Emeryville Marina.
[f] Fish collected directly south of Standard Oil dock, to south side of Richmond-San Rafael Bridge.
[g] Fish collected in approximately 10-15 feet of water east of the mouth of the Petaluma River.

River, north of Moss Landing, Monterey Bay, produced only 4 flounders. None of these fish was running ripe. None of the females from any field collections was intensely swollen, which is characteristic of ready-to-spawn fish.

MORPHOMETRIC ANALYSIS RESULTS

GONADOSOMATIC INDEX (GSI)

Figure 2 indicates the GSI's measured in 2-4 year old flounder collected at each site (Table 2). Preliminary statistical analysis indicated that the variance for each group was dependent on the mean ($r = 0.96$). In order to achieve homogeneity of error in the variance, the GSI data were log transformed. A two-way ANOVA indicated a significant interaction term ($p= 0.002$). Figure 3 suggests this interaction is primarly due to Berkeley and in particular, a single 4-year-old fish. Interaction measures parallel response. We need more 4-year-old-fish in order to draw valid conclusions regarding GSI trends in the 4-year-old group. In order to examine the simple main effects of site upon GSI, a one-way ANOVA was performed on 3-year-old fish from all sites. Some differences were found between sites ($p = 0.11$). A multiple comparison of means indicated significant differences between fish collected in San Pablo Bay and Moss Landing (Table 2). Three year old flounder collected from San Pablo Bay had the highest GSI's.

AGE/STANDARD LENGTH RELATIONSHIPS

Table 3 shows the mean standard length (SL) for each year class of flounder at each collection site. An analysis of covariance on untransformed data indicates that the slopes of the growth rates for Moss Landing, Berkeley,

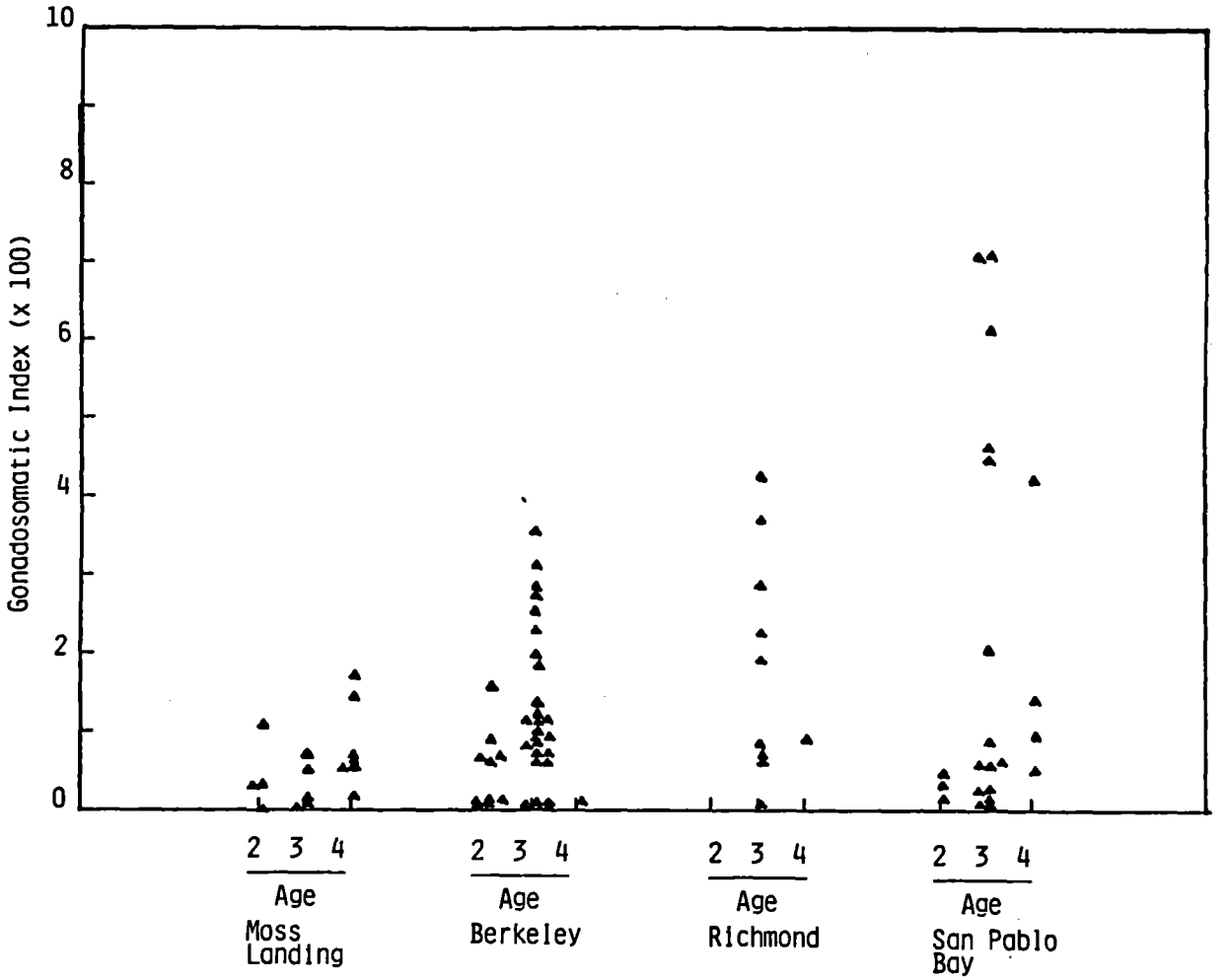


Figure 2. Frequency distribution of gonadosomatic indices for P. stellatus 2-4 years old collected October, 1982, through February, 1983.

and North Bay fish (Richmond fish pooled with San Pablo fish) were parallel ($p = 0.86$). There were significant differences in SL among collection sites ($p = 0.002$). A multiple comparison test of adjusted means indicated Moss Landing fish were significantly larger than San Francisco Bay fish ($p \leq 0.01$). A "t" test comparing the SL of Berkeley and San Pablo Bay one year old fish indicated Berkeley fish were significantly smaller ($p = 0.01$).

Table 2. Gonadosomatic Index (gonad wt/body wt x 100) for starry flounders age 2-4 years collected October, 1982, through February, 1983. Means and standard deviations.

Collection site	Age 2	Age 3[a]	Age 4
Moss Landing (ML)	0.44 ± 0.47 (4)[b]	0.30 ± 0.30 (5)	0.83 ± 0.55 (7)
Berkeley (BK)	0.50 ± 0.49 (10)	1.3 ± 1.0 (27)	0.11 (1)
Richmond (RH)	--	1.9 ± 1.5 (9)	0.92 (1)
San Pablo Bay (SP)	0.32 ± 0.17 (3)	2.3 ± 2.7 (15)	1.8 ± 1.7 (4)
San Francisco Bay (combined)	0.46 ± 0.44 (13)	1.7 ± 1.8 (51)	1.4 ± 1.5 (6)

[a] Results of Tukey-Kramer multiple comparison of log mean GSI (3 yr old fish):

ML BK RH SP

Mean GSI of sites underlined are not significantly different ($p \geq 0.05$).

[b] Number of fish.

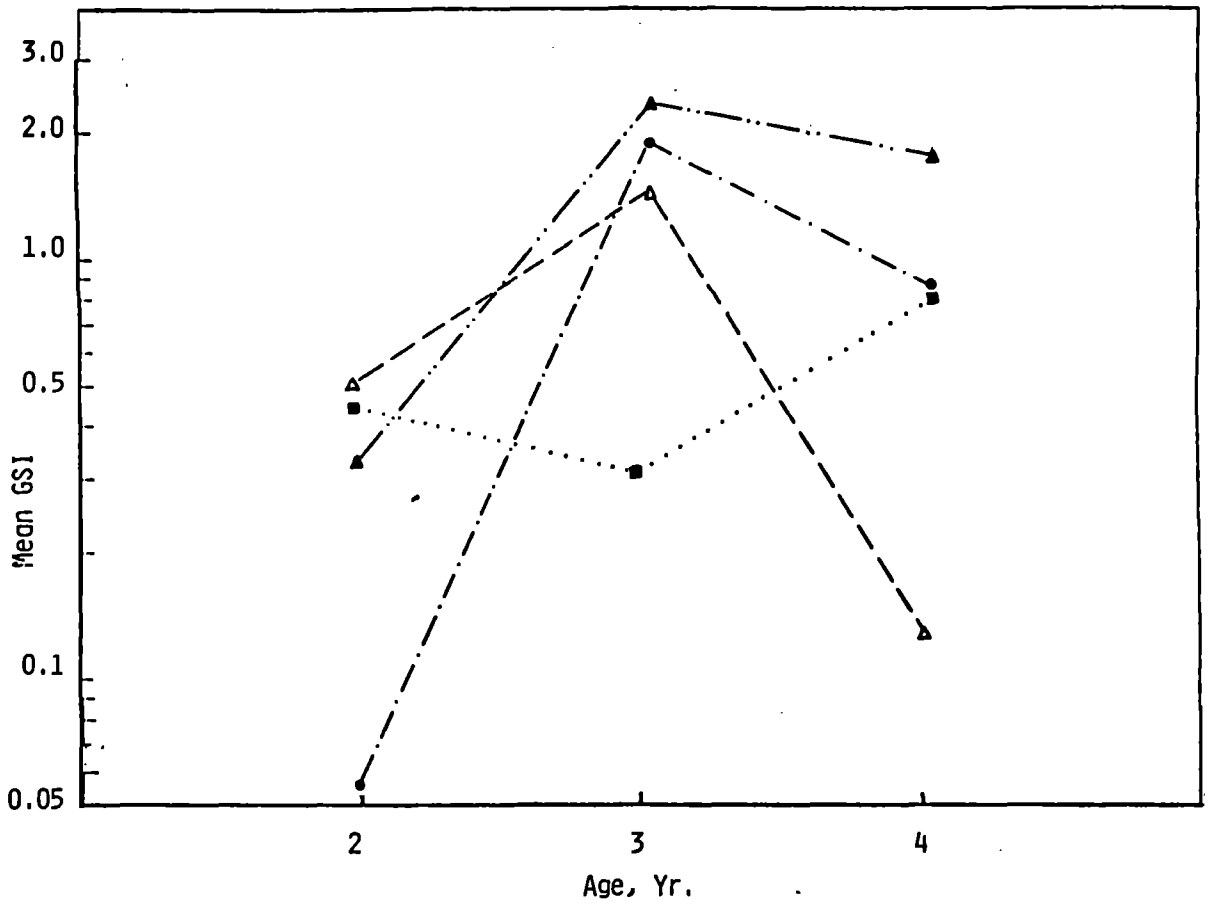


Figure 3. Log mean gonadosomatic indices for P. stellatus 2-4 years old collected October, 1982, through February, 1983. Collection site is indicated along with the number of fish measured for each age and site.

HEPATOSOMATIC INDEX (HSI)

A one-way ANOVA indicates that there are significant ($p < 0.05$) differences in HSI between all collection sites (Table 4). Moss Landing fish had the smallest average liver sizes in relation to body size. Richmond and San Pablo Bay fish had similar HSI's, and the largest average liver size relative to body size. Berkeley fish had significant relationships between MFO

Table 3. Results of tests for differences in P. stellatus standard length (SL) observed in year classes 1-4 collected October, 1982, through February, 1983. Means ± one standard deviation are given (n = number of fish).

Year class	ML	BK	RH	SP	NB
1	13 ± 1.1 (4)	9.5 ± 1.8 (22)	--	11 ± 3.3 (52)	--
2	23 ± 6.1 (8)	25 ± 4.8 (12)	--	23 ± 4.8 (10)	--
3	30 ± 6.4 (9)	28 ± 2.9 (36)	29 ± 4.2 (11)	28 ± 3.2 (22)	28 ± 3.7 (33)
4	40 ± 6.5 (7)	29 ± 2.6 (4)	34 ± 4.9 (2)	31 ± 3.4 (6)	32 ± 3.7 (8)

Results of multiple comparison of adjusted SL means:

ML BK NB

Adjusted mean SL of sites underlined are not significantly different ($p \leq 0.05$).

ML = Moss Landing

BK = Berkeley

RH = Richmond

SP = San Pablo Bay

NB = North Bay (SP + RH)

Table 4. Hepatosomatic (liver weight/body weight x 100 = HSI) for P. stellatus collected October, 1982, through February, 1983. Correlation coefficients were determined between HSI and hepatic mixed function oxidase activity measures.

	ML[a]	BK[b]	RH[c]	SP[d]	NB[e]
Liver index (100X) mean ± one std. dev.	1.4 ± 0.77	1.6 ± 0.51	2.0 ± 0.16	1.9 ± 0.66	1.9 ± 0.62
Median	1.2	1.4	1.9	1.7	1.9
Number of fish examined	20	19	4	30	34
One-way ANOVA test results:	All sites (ML, BK, RH, SP): p = 0.024				
	Correlation coefficients				
Hepatosomatic Index vs:					
MFO specific activity[h] (p mol mg^{-1} min^{-1})	0.056	-0.43[f]	-0.25	-0.097	-0.10
Total hepatic MFO Activity[h] (m mol min^{-1})	0.18	0.36	0.50	0.42[f]	0.42[g]
MFO activity g^{-1} fish[h] (m mol min^{-1} $(g\ fish)^{-1}$)	0.31	-0.038	0.27	0.30	0.30[f]

[a] ML = Moss Landing

[b] BK = Berkeley

[c] RH = Richmond

[d] SP = San Pablo Bay

[e] NB = North Bay (SP + RH)

[f] Significant correlation ($p < 0.05$).

[g] Significant correlation ($p < 0.01$).

[h] All MFO values were log transformed (ℓnx).

measures and HSI. Higher MFO specific activity was significantly related to smaller livers ($p < 0.05$), and higher total hepatic MFO activity was related to larger livers ($p < 0.05$) in Berkeley fish (data not shown).

MIXED FUNCTION OXIDASE CORRELATIONS WITH MORPHOLOGICAL MEASUREMENTS

MFO SPECIFIC ACITIVTY

A total of 70 fish were analyzed for hepatic MFO activity by the aryl hydrocarbon hydroxylase (AHH) assay. The assay measures the conversion of benzo(a)pyrene to the 3-OH product by microsomal protein *in vitro*. Our methodology for this assay is outlined in Spies *et al*. (1982). The results are expressed as picomoles of 3-OH benzo(a)pyrene • mg microsomal protein^{-1} • min^{-1}.

Figure 4 shows the distributon of MFO specific activities measured at each collection site. Mean MFO specific activity and the number of fish analysed at each site are given in Table 5a. One-way ANOVA and Tukey-Kramer multiple comparison procedures indicate significant differences in MFO specific activity among collection sites and among collection sites within San Francisco Bay. Berkeley fish had the highest average MFO specific activity. Richmond and San Pablo Bay fish had the lowest.

Correlation coefficients between body weight and three MFO measures were determined (Table 5b). MFO specific activity represents enzyme activity per mg of liver microsome protein. Total hepatic MFO activity represents the total MFO enzyme activity of a fish liver. The MFO activity g^{-1} fish represents the total hepatic MFO activity divided by body weight of the fish.

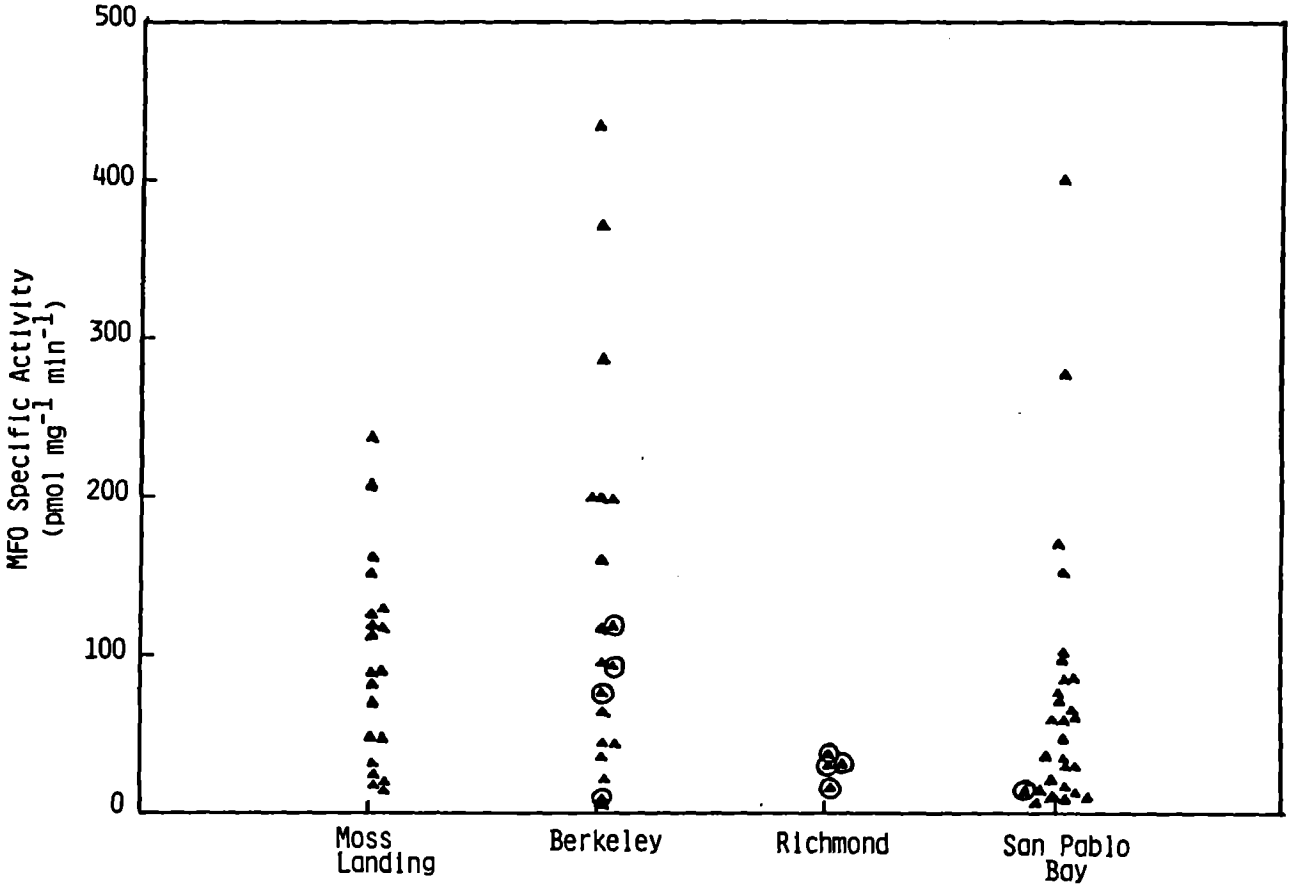


Figure 4. Frequency distribution of MFO specific activities measured in P. stellatus collected October, 1982, through February, 1983. Spawning females are circled. Tests for skewness and kurtosis indicated that MFO specific activites were not normally distributed.

A moments test for skewness and kurtosis indicated that MFO values were not normally distributed. A log-normal transformation (ℓn x) resulted in a normal distribution, so transformed MFO data were used for all subsequent correlation analyses. Berkeley fish had a significant correlation between total hepatic MFO activity and body size ($r = 0.82$). North Bay fish had a significant negative correlation between MFO specific activity and body size.

Table 5. (a) Mixed function oxidase (MFO) activity for P. stellatus collected October, 1982, through February, 1983; (b) Correlation coefficients for MFO measures versus body size (wet wt., g).

Collection site:	ML	BK	RH	SP	NB[a]
a. MFO Measures: Data Summary:[b]			Mean ± one Std. Dev.		
MFO specific activity[c] (p mol mg^{-1} min^{-1})	95 ± 63	130 ± 121	30 + 9	71 + 86	56 ± 59
Total hepatic MFO[d] activity (m mol min^{-1})	18 ± 17	19 ± 20	10 ± 5.1	8.0 ± 10	8.3 ± 9.9
MFO activity g^{-1} fish (η mol min^{-1} g $fish^{-1}$)	27 ± 23	25 ± 20	9.7 ± 3.9	19 ± 25	18 ± 23
b. MFO Measures Correlation Matrix: Variable vs body size					
MFO specific activity[g]	-0.34	-0.13	0.17	-0.42[e]	-0.33[e]
Total hepatic MFO activity[g]	0.38[e]	0.58[f]	-0.64	0.098	0.25
MFO activity g^{-1} fish[g]	-0.20	-0.15	0.29	-0.24	-0.15
Number of fish analyzed	20	19	4	30	34

[a] Richmond and San Pablo Bay data pooled.

[b] Tukey-Kramer multiple comparison procedure results:

MFO Specific Activity: BK ML NB

Total Hepatic MFO Activity: BK ML NB

mean MFO measures of fish collected at underlined sites are not significantly different ($p \leq 0.05$).

[c] Significant differences between ML, BK, NB means ($p \leq 0.05$) (one-way ANOVA).

[d] Significant differences between ML, BK, NB means ($p \leq 0.05$) (one-way ANOVA).

[e] Significant correlation ($p \leq 0.05$).

[f] Significant correlation ($p \leq 0.005$).

[g] All MFO values were log-transformed (ℓnx).

LABORATORY SPAWNING

All fish captured during the December-February collections were returned to the laboratory live and segregated into holding tanks by collection site. Flounders larger than about 300-400 grams were measured, weighed and then started on a course of hormone injections to induce the final stages of gonad maturation and spawning in the laboratory. Fish were injected daily with freezed-dried carp pituitary dissolved in normal physiological saline, to give a dose of 1 mg pituitary $\cdot$ kg^{-1}.

Of the 79 fish injected, 15 were females that eventually spawned (Tables 6 and 7). We had numerous males to choose from to fertilize the eggs but decided to use only 2 males (MSB 2478, 2531). We mainly used MSB 2478 which gave the highest rate of fertilization in a trial with two other males using the same female. This was done because we wished to concentrate our efforts on possible effects of pollutant body burdens on oocyte quality. We will perhaps treat male variability in the second year, but we decided to keep the male factor constant in the first year.

Each successfully spawned female was stripped of eggs on at least two and usually three separate days within the period of a week. Eggs were examined for fertilization success 24 h after mixing of eggs and sperm. Fertilization success was based on the presence of a fertilization membrane (Fig. 5). The results of the spawning with the highest fertilization success for each female are shown in Table 8. The capacity of oocytes to be successfully fertilized was in the order San Pablo Bay > Richmond > Berkeley. Failed embryos would sink to the bottom of their incubation beakers and were collected daily and preserved. At hatching, both normal and abnormal larvae (Fig. 6) were harvested and preserved.

Table 6. Summary of female P. stellatus collected during December, 1982, and January and February, 1983, and induced to spawn in the laboratory.

Site	Collection date	MSB #	Start inject date	Spawning dates	Number of spawnings	Wt. of fish (g)	Length of injection[a] (d)	Vol[b] injected ml
Berkeley	12/14/82	2457	12/16	12/27-1/10	4	2690	11	2.7
	1/17/83	2551	1/18	1/31-2/1	2	1055	13	1.1
		2553	1/18	2/1 -2/17	3	1012	14	1.0
		2556	1/18	1/27-2/1	3	660	9	0.7
	2/23/83	2612	2/24	3/9 -3/11	2	580	13	0.6
Richmond	12/14/82	2469	12/16	1/3 -1/10	4	1470	17	1.5
	1/17/83	2567	1/18	1/28-1/31	3	1025	10	1.0
		2568	1/18	1/27-2/1	4	1206	9	1.2
		2569	1/18	1/27-2/1	3	1240	9	1.2
		2570	1/18	2/13-2/17	3	1045	26	1.0
San Pablo Bay	12/14/82	2479	12/16	1/9 -1/19	3	685	24	0.7
		2480	12/16	1/16-1/19	2	2070	31	2.1
		2484	12/16	1/23-1/26	2	490	38	0.5
	2/3/83	2596	2/4	2/23-3/2	3	712	19	0.7
		2600	2/4	2/19-2/22	3	853	15	0.9

[a] Until first spawning; number of days also equals the total number of mg/kg of pituitary that each fish received.

[b] Carp pituitary; 1 mg pituitary/ml physiological saline.

Table 7. Summary of *P. stellatus* developmental success by site.

Site	MSB number	Number of eggs used	Developmental success (mean %)		
			Fertilization	Hatching	Normal Larvae[a]
Berekeley	2457[b]	6600	16.8	4.0	86.4
	2551[b]	10,600	31.6	37.5	52.8
	2553	6710	84.5	52.5	83.4
	2556[b]	5500	23.0	0.8	57.1
	2616	8100	83.0	40.9	81.5
Mean ± S.D.		7500	47.8	27.1	72.2
		± 1900	± 33.3	± 23.3	± 16.0
Richmond	2469[b]	5700	50.0	48.2	74.3
	2567[b]	11,100	43.0	47.7	76.8
	2568[b]	1010	72.0	30.2	83.3
	2569[b]	7710	45.0	18.3	85.7
	2570	11340	56.6	22.8	87.4
Mean ± S.D.		7370	53.3	33.4	81.5
		± 4270	± 11.7	± 13.9	± 5.7
San Pablo Bay	2479	7690	52.8	9.0	79.7
	2480	9810	75.3	32.2	69.6
	2484	17,500	71.1	20.2	39.1
	2596[b]	6620	53.5	26.5	63.1
	2600	6480	72.2	8.5	46.2
Mean ± S.D.		9620	65.0	19.3	59.5
		± 4600	± 10.9	± 10.5	± 16.7

[a] Significant differences between sites ($p < 0.10$).
[b] MFO specific activity determined within 30 days of capture.

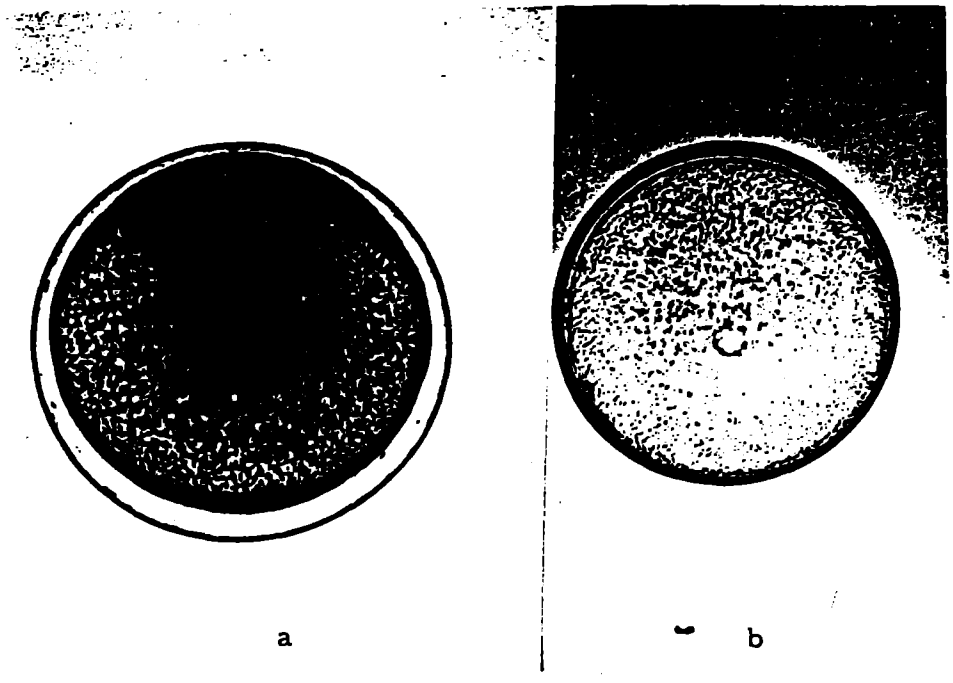


Figure 5
a. Fertile starry flounder egg

b. Unfertilized starry flounder egg

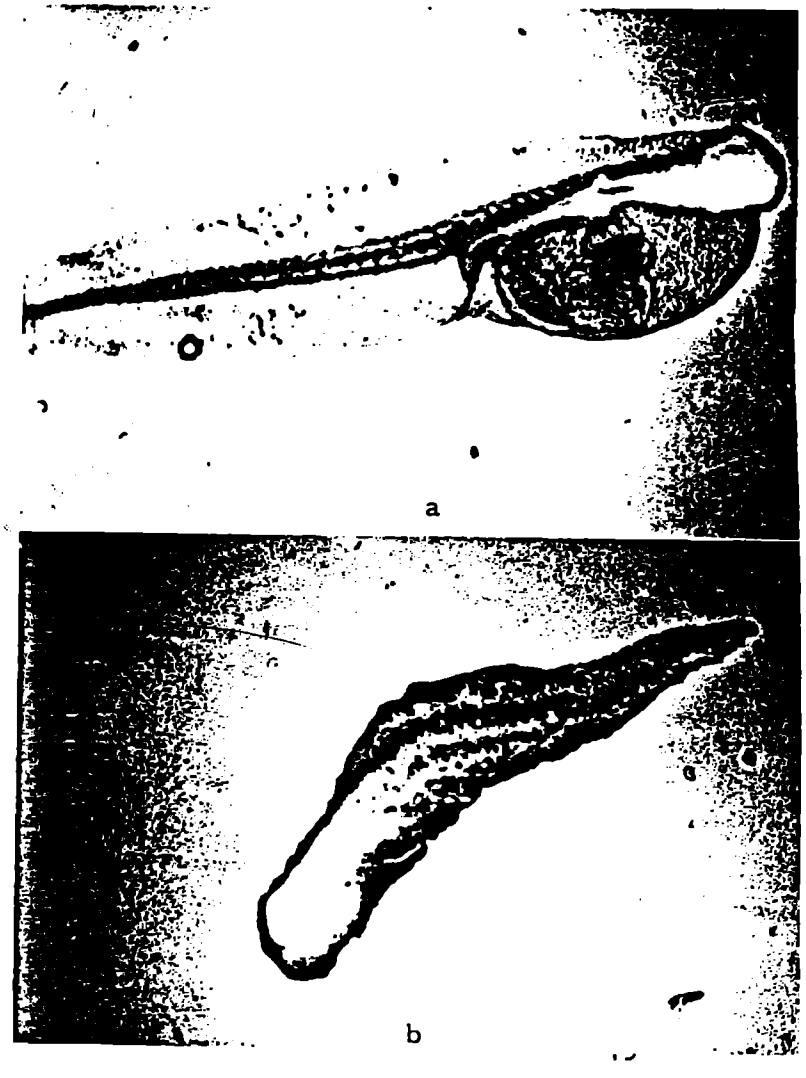


Figure 6

Newly hatched starry flounder larvae:

a. Normal larva

b. Abnormal larva with deformed fin fold, reduced yolk, and contorted spine

Table 8. Concentration of chlorinated hydrocarbons extracted from starry flounder oocytes. Included are summaries of developmental success measured in the oocytes. MFO specific activities of female flounder producing oocytes are also included. (p mol mg^{-1} min^{-1})

MSB No.	Collection site	Percent			MFO Spec. activity	oocyte concentration, ppb						
		Fert	Hatch	Normal larvae		Total PCB	Total DDT	Total chlorinated hydrocarbons	Hepta-chlor	Hepta-chlor-expoxide	β-BHC	Lindane
2457	BK	17	4.0	86	88	33	10	47	BD	BD	BD	0.40
2551	BK	32	37	53	110	27	6.0	35	BD	0.20	0.05	BD
2553	BK	84	52	83	--	58	12	75	0.02	BD	0.04	0.06
2556	BK	23	0.81	57	130	28	7.0	39	0.03	BD	0.05	1.8
2469	RH	50	48	74	38	29	12	36	BD	0.20	BD	BD
2567	RH	43	48	77	46	12	3.5	16	0.31	0.05	0.02	0.040
2568	RH	72	30	83	20	20	7.6	29	0.04	BD	BD	0.30
2569	RH	45	18	86	39	7.0	2.7	10	0.24	0.30	0.04	BD
2596	SP	53	26	63	15	20	7.2	33	0.01	0.10	0.01	0.10

BK = Berkeley

RH = Richmond

SP = San Pablo Bay

BD = Below detection limit

MFO specific activity was measured within 30 days of capture in 8 of the female flounder spawned. The correlation between hepatic MFO specific activity and percent fertilization was highly significant (Fig. 7). Percent hatching and percent normal larvae did not correlate well with MFO specific activity. Significant differences among collection sites were found for percent normal larvae (Table 8).

POLLUTANT BODY BURDENS

Analyses of pollutant body burdens were begun after the enzyme assays and embryo and larval development work had been completed and the data analyzed.

Tissues to be analyzed for organic pollutants were cut up and dried by mixing with 25 g or more of anhydrous Na_2SO_4. They were further macerated and extracted by addition of acetonitrile and treatment in a high speed tissue homogenizer (Polytron). The acetonitrile was decanted and the procedure repeated twice. The final extract was separated from the solid phase by centrifugation at 5000 rpm for 15 min. The extract volume was made up to 100 ml with acetonitrile and placed overnight in a refrigerator at 5 to 10°C. This generally resulted in a flocculent lipid material coming out of solution. Fifty milliliters of the extract were withdrawn, most of the lipid flocculent was avoided, and reduced in volume to 4 ml in a rotary evaporator.

Sample cleanup was accomplished with C_{18} and amino columns (Baker Chemical Co.) in series. The C_{18} column was pre-cleaned with acidified methanol followed by rinses of methanol and acetonitrile. The amino column was pre-cleaned with one rinse of acetonitrile. The four milliliter sample was loaded on the column and pulled through by suction. This was followed by two 0.5-ml rinses of acetonitrile. The C_{18} column was removed and stored

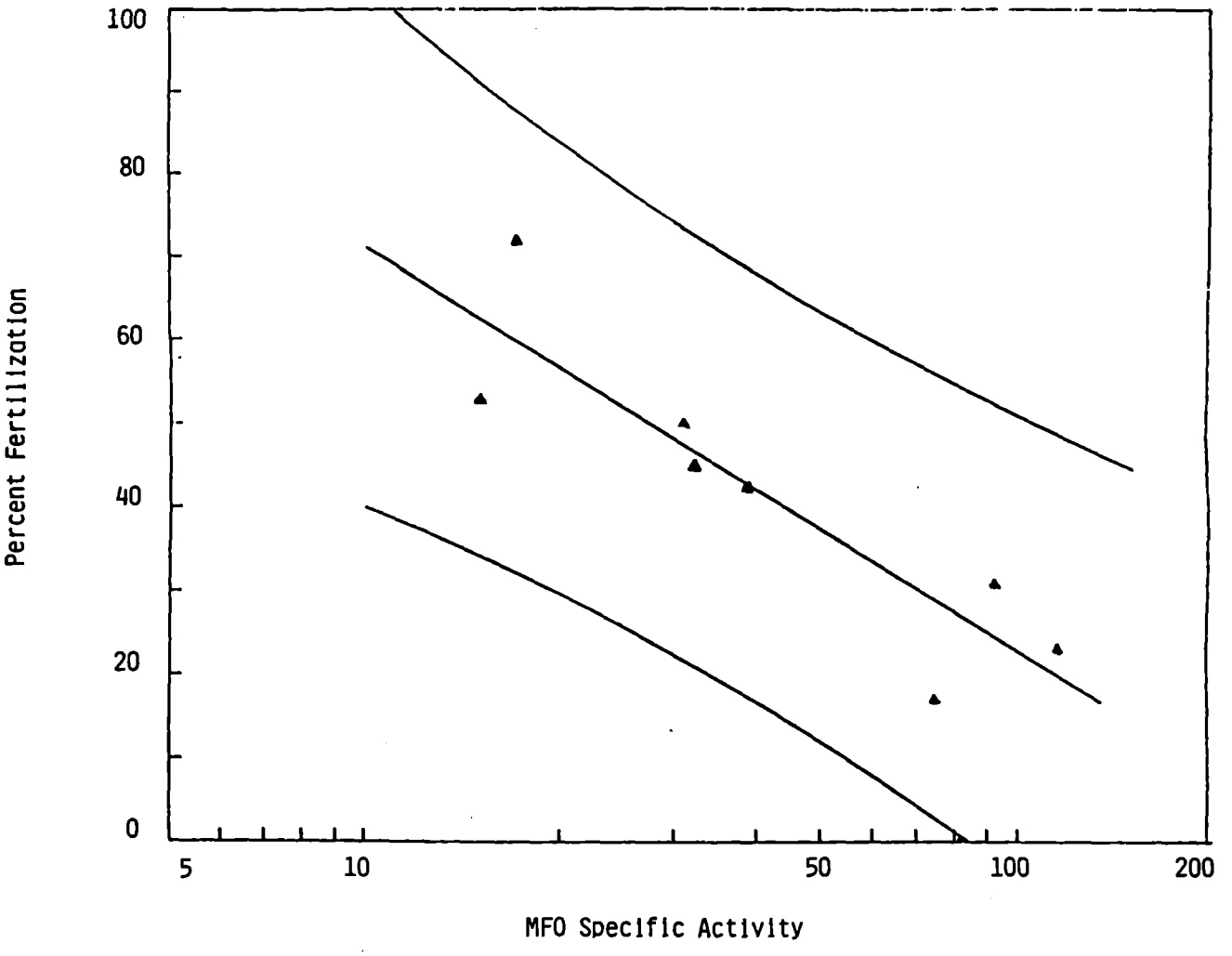


Figure 7. MFO specific activity versus percent fertilization for P. stellatus females collected in San Francisco Bay during December, 1982, and January and February, 1983. The correlation coefficient (r) is -0.89, and the equation for the line is $y = 70 - 21 \ln x$ ($F = 23$ with (1,6) D.F.; $p = 0.003$). 95% confidence intervals about the line are shown.

under clean refrigerated conditions for later elution of saturated hydrocarbons. Finally, the sample volume was reduced to a volume suitable for gas chromatography (<0.5 ml).

Organic pollutants of interest were then assayed by computer assisted gas chromatography. We used a Hewlett-Packard 5880 Gas Chromatograph equipped with a ^{63}Ni electron capture detector, a capillary injector and a 30-m fused

silica capillary column (DB-1). Polychlorinated biphenyls (PCBs) and pesticides have been identified by comparison of retention times with those of standards.

A computer program performed data analysis on raw instrument input stored in a Hewlett Packard 3350 automated laboratory data system. Program input parameters include original tissue weight, injection volume and response factors. Individual chlorinated hydrocarbons were resolved against a background of a complex mixture of halogenated and oxygenated compounds, so quantification here is based on a mode of peak skimming that goes from valley to valley. Our values, therefore, might be expected to be conservative for some compounds. Detection limits under these conditions are estimated to be about 100 parts per trillon.

A representative gas chromatograph of an oocyte sample is shown in Fig. 8. Results of analyses by electron capture of oocytes from spawning females are given in Table 8. Total PCB is the sum of all peaks tentatively identified as chlorinated biphenyls (22 compounds that were the predominant peaks in the Arochlor 1254 standard). Only total DDT (DDT + DDD + DDE) is shown as some interconversion occurs during gas chromatography.

It can be seen that total identified chlorinated hydrocarbons range from approximately 10 to 75 ppb, total PCB from 7 to 58 ppb and total DDT from 3 to 12 ppb in the oocytes of spawning females. For purposes of comparison, the collection site, measures of fertilization success and MFO specific activity are also shown in Table 8.

In Table 9 correlation coefficients of MFO specific activity, total liver activity and chlorinated hydrocarbons in oocytes with measures of fertilization success are given. There is also a correlation matrix for MFO and the various classes of chlorinated hydrocarbons.

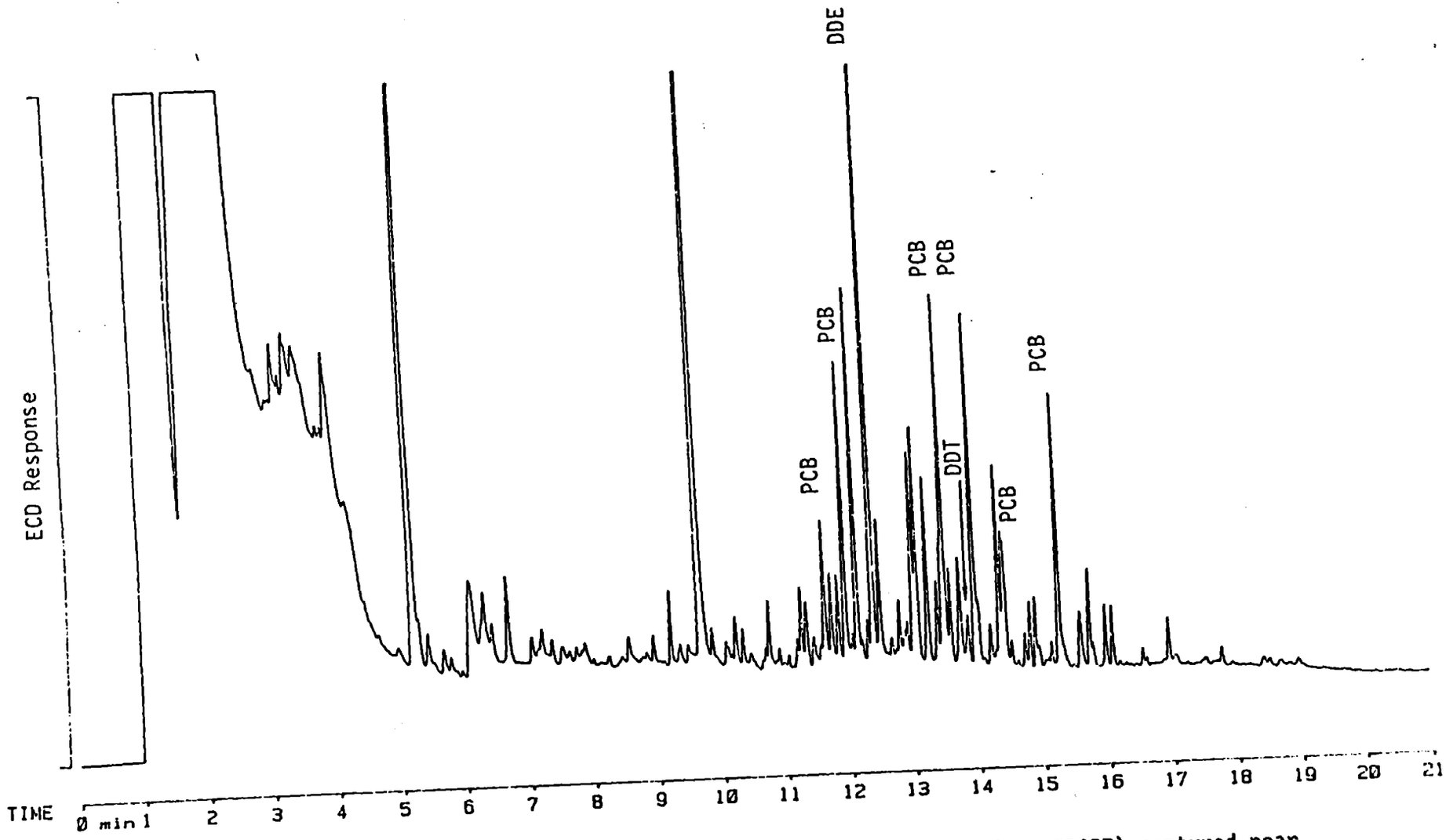


Figure 8. Gas chromatogram of oocyte extract from female starry flounder (MSB #2457) captured near Berkeley on December 14, 1983, spawned in the laboratory on December 27, 1983. Total identified chlorinated hydrocarbons: 47.5 ppb.

Table 9. Correlation coefficients of MFO specific activity, total liver activity and concentration of chlorinated hydrocarbons in oocytes versus developmental success measures of starry flounders captured in San Francisco Bay.

	Fertilization success (%)	Hatching success (%)	Normal laval (%)
ℓn MFO specific activity (MFO) (p mol $mg^{-1}min^{-1}$)	-0.87[c]	-0.42	-0.35
ℓn Total hepatic MFO activity (TLA) (m mol min^{-1})	-0.83[c]	-0.30	-0.04
PCBs (ppb wet wt.)	0.31	0.23	0.05
ΣDDT (ppb wet wt.)	0.27	0.23	0.13
Total chlorinated hydrocarbon	0.31	0.75[c]	0.04

Correlation matrix

	PCBs	ΣDDT	Total chlorinated hydrocarbons
ln MFO	0.51[a]	-0.05	0.45
ln TLA	0.69[b]	0.39	0.61[a]
PCB		0.49	0.14
DDT			0.61[a]

[a] $p < 0.1$

[b] $p < 0.05$

[c] $p < 0.01$

The very significant correlation of fertilization success with MFO specific activity has already been discussed but there was also a correlation of total hepatic MFO activity with fertilization success ($r = -0.83$, $p < 0.01$). Of further interest are the relationships (1) between PCBs in oocytes and MFO specific activity ($r = 0.51$, $p < 0.1$) and total hepatic MFO activity ($r = 0.69$, $p < 0.05$) and (2) total chlorinated hydrocarbons and total hepatic MFO activity ($r = 0.61$, $p < 0.1$). When comparing the concentrations of chlorinated hydrocarbons in oocytes to reproductive success a relationship is seen between hatching success and total chlorinated hydrocarbons ($r = 0.75$, $p < 0.07$). Obviously these results are of great interest and require further investigation, but caution should be exercised in drawing broad conclusions from so few data.

Rather than make comparisons on the basis of individual fish it is useful to compare mean values of parameters of spawning females by site. Four of the spawning females were from Berkeley and these are compared in Table 10 with four from Richmond. An additional spawning female came from San Pablo Bay, but it is not included in the table. It can be seen that in every case the mean enzymatic activities and contaminant levels are higher and the measures of reproductive success are lower in Berkeley females. Despite the small number of fish tested, "t" tests indicate that several of these parameters are significantly different between sites. This tends to support the thesis that higher contaminant levels are associated with reduced reproductive success and elevated levels of MFO activity.

DISCUSSION

The most signficant finding of this study was the reduction of fertilization success in female starry flounders having higher MFO specific

Table 10. Hepatic enzyme activities and contaminant concentrations in oocytes and reproductive success in spawning females by collection sites, mean ±.

	Number of fish in parentheses	
	Berkeley (n = 4)	Richmond (n = 4)
MFO specific activity ($p\ mol\ mg^{-1}\ min^{-1}$)	109[c]	36
Total hepatic MFO activity ($m\ mol\ min^{-1}$)	51[c]	15
Total PCBs (ppb, wet weight basis)	36.7[a]	17.1
Total DDT (ppb, wet)	9.1	6.5
Total chlorinated hydrocarbons (ppb, wet weight basis)	49[b]	23
% fertilization	39	52
% hatching	23	36
% normal larvae	70[a]	80

[a] $p < 0.10$.

[b] $p < 0.05$.

[c] $p < 0.005$.

activities. While further data will be sought to verify this relationship, the implication for reproductive success of starry flounder populations are important. Changes in survival of fish during early life history stages will have important impacts on the size of a given year class of fish.

We have framed three alternative hypotheses, each of which provides a mechanistic interpretation of how increased levels of MFO might be related to decreased fertilization success.

Hypothesis I: Pollutant metabolites, formed at a greater rate in induced fish, are accumulating in the oocytes and interfering with successful fertilization.

Hypothesis II: Increased MFO activity merely reflects high levels of parent (pollutant) compounds in induced fish and it is the parent compounds that are causing the gamete damage.

Hypothesis III: Increased MFO activity of induced fish alters hormone levels (e.g., estradiols) and the altered hormonal balance in turn results in the production of oocytes with a reduced capacity for fertilization and successful hatching.

These hypotheses are not necessarily mutually exclusive and the relationship seen could conceivably be based on a combination of the above explanations.

There is good circumstantial evidence for proposing Hypothesis I, that the observed oocyte damage is due to pollutant metabolites. Varanasi et al. (1982) have shown that English sole force fed radiolabeled benzo[a]pyrene shortly before spawning subsequently had various metabolic products (including phenols, quinones, 7,8-dihydro-7,8-dihydroxy- and 9,10-dihydro-9,10-dihydroxy-benzo[a]pyrene) in their livers and gonads. Less than 10% of the radiolabel was incorporated into excretable products (glucuronides and sulfates), while benzo[a]pyrene intermediates were bound to hepatic and gonadal proteins and

DNA. Starry flounder induced with 3-methylcolanthrene have been shown to bind actively radioabeled benzo[a]pyrene to a greater extent than English sole (Varanasi et al., 1980). From the extensive mammalian literature it is known that these metabolic products by virtue of their binding to DNA molecules can cause a variety of cellular disorders including carcinogenic, teratogenic and mutagenic events. It is generally accepted that the carcinogenicity of xenobiotics correlates roughly with their degree of covalent binding to DNA (Buty et al., 1976; Kouri et al., 1973; Philips et al., 1978). Among the described effects for polycyclic aromatic hydrocarbons (PAH) in mammals is oocyte toxicity (Felton et al., 1980; Felton and Dobson, 1983). Our first year results suggest this phenomenon occurs in fish from polluted environments. Hose et al. (1981) demonstrated that flathead sole fed 4.0 mg of benzo[a]pyrene prior to spawning exhibited a significantly lower hatching success than control fish.

The second hypothesis, that the parent compounds are responsible for the observed damage, is the logical complement to Hypothesis I and should also be considered. Further work on levels of non-chlorinated hydrocarbons and on pollutant concentrations in the livers of these fish may allow more definitive conclusions to be drawn about any direct relationships between levels of parent hydrocarbons and reproductive effects.

The third hypothesis, that the oocyte damage results from an altered hormone balance, is similar to the first hypothesis in that there is some support in the mammalian literature for such a relationship. For instance, inhalation of para-xylene by gestating rats can cause embryo toxicity, and such toxicity is accompanied by decreases in progesterone and 17β-estradiol (Ungvary et al., 1981). It is thought that exposure to inducing aromatic hydrocarbons results in an increase of P-450 enzymes which in turn produces increased rates of hormone metabolism. One of the functions of MFO in fish is

catabolic regulation of hormones, and it seems likely that sex-related and seasonal MFO differences (Walton et al., 1978; Stegeman and Chevion, 1979; Stegeman, 1980) seen in fish populations reflect this functional role. Because aryl hydrocarbon hydroxylase (AHH) activity, most commonly used to monitor levels of MFO in fish is a P-450 linked enzyme as are the steroid metabolism enzymes, it is not yet clear to what degree pollutant-induced increases in P-450 may effect steroid metabolism. This type of question is, however, receiving a great deal of attention from biochemists such as Dr. John Stegeman and Dr. James Bend and others as they try to unravel mechanisms of P-450 function in fish.

Another of the interesting relationships in the developmental data is that fertilization success, hatching success and the number of normal larvae, although cumulative in total reproductive success, appear to be independently distributed both within individual fish and among populations. Perhaps, different pollutants exert their influence at different developmental stages. While there are insufficient results yet to justify a hypothesis, we speculate that the central San Francisco Bay populations, because of their higher MFO activities, are probably exposed to high levels of PCBs and petroleum hydrocarbons (inducers of MFO). The San Pablo Bay populations, because of their poor hatching success, are probably exposed more to pesticides (non-inducers of MFO) from the Sacramento and San Joaquin Rivers. Petroleum hydrocarbons and their water-soluble metabolites may be affecting developing oocytes in the maturing females from Berkeley while in the San Pablo Bay fish less easily-metabolized chlorinated pesticides are sequestered in the yolk and exert toxic effects later as the developing embryos and larvae draw on their yolk reserves. This hypothesis will be resolved by further chemical analysis of gonads, eggs and liver tissue of Central and North Bay populations. In the

Baltic, von Westernhagen et al. (1981) found that concentrations of PCB of greater than 120 ppb in the eggs result in reduced survival of the larvae.

First year class fish might be important indicators of population stress. Significant differences in mean standard length (SL) between different collection sites were observed. First year class fish may have limited ranges of movement compared to older fish and may be better indicators of site specific pollutant effects. Though MFO activities were not measured in first year class fish this year, we plan to do so during the coming sampling season. We will try to correlate SL with MFO activities measured in these young fish. Another explanation of differences in size between Central and North Bay year-1 fish is that North Bay fish may be better nourished. The fact that the hepatosomatic index of North Bay fish was higher than that of Berkeley fish and that the liver is a storage organ, suggests that North Bay fish may be very well nourished. San Pablo Bay is generally regarded as an extremely productive area within San Francisco Bay.

The Berkeley site may represent an environment polluted by MFO inducers and one in which starry flounder populations may be affected by sublethal levels of pollutants. The total hepatic MFO activity in Berkeley fish was significantly related to HSI and to body size. A possible interpretation of these relationships may be that older (larger) fish have resided in the Berkeley area for a longer period of time and have higher total MFO hepatic activities and larger livers relative to body size. The fact that Berkeley 1st year class fish were significantly smaller, coupled with lower fertilization success and a lower gonadosomatic index, indicates that Berkeley starry flounder populations are probably under pollutant stress. Moss Landing fish also had relatively high MFO activity measures and low gonadosomatic indices. Too few first year class fish were collected at Moss Landing to draw conclusions about differences in standard length.

PROJECT GOALS IN 1983-1984

One of the primary goals of the project in the coming year will be to continue to explore the relationship between mixed function oxidase activity in fish and fertilization and hatching success. We think that the decrease in fertilization and hatching success with increasing MFO activity within the induced mid-Bay population are the most meaningful and interesting findings of the first year's work. Much of our work in the coming year is designed to support this part of the project and explore further this relationship.

Other goals include determining if there is a relationship between fertilization, reduced hatching success or abnormal larvae and pollutant body burdens, collecting more year-1 fish to see if the 1982-1983 year class of starry flounder from the Berkeley area continues to show stunted growth, and to carry out more detailed chemical characterizations of tissue pollutants.

Our general study strategy during the second year will be similar to that of the first year. This strategy is detailed in Table 11. Again, our major field efforts will be carried out in October/November and then in December, January and February. We will again make morphometric measurements and measurements of reproductive success. The tissue chemistry, which started in the second half of this year, will continue as a large part of the program in the second year. We feel that more data are needed before any conclusions can be reached in regard to relationships between pollutant body burdens and the biological measures. Examples of trends that requires further data are (1) the correlation between PCBs and total hepatic MFO activity, and (2) between total chlorinated hydrocarbons and hatching success.

An additional aspect of our work planned for the coming year involves measurements of rates of hormone metabolism and blood-hormone levels. This is an attempt to resolve Hypothesis III, that the reduced fertilization success

Table 11. Data and experiments for major field efforts in 1983-1984.

Tasks	October/November	December/January/February
• Morphological measurements		
- gonad and liver weights	x	x
- standard length	x	x
- age determinations	x	x
• MFO enzyme assays	x	x
• Steroid metabolism	x	
• Blood steroid levels	x	
• Spawning		
- percent fertilization		x
- hatching success		x
- percent normal larvae		x
• Tissue chemistry		
- chlorinated hydrocarbons/pesticides	x	x
- petroleum hydrocarbons	x	x

of female fish with high MFO activity is an effect mediated through altered hormone metabolism. There are two aspects to this planned work and both involve cooperative efforts with other scientists that have expressed an interest in our results. First, to see if rates of estradiol metabolism in females are affected by high levels of MFO, as determined by aryl hydrocarbon hydroxylase (AHH) measurements, we plan to send hepatic microsomal fractions of spawning females to Dr. John Stegeman of Woods Hole Oceanographic Institution. In Dr. Stegeman's laboratory, the microsomal fractions will be assayed for rates of estradiol metabolism. Rates of estradiol metabolism will then be compared with AHH activity, measures of reproductive success and pollutant body burdens. Second, to see if levels of circulating hormones are also affected, blood samples will be taken from each fish and sent to Dr. Peter Thomas at the University of Texas. Dr. Thomas will assay the samples for estradiol, testosterone and 17-ketotestosterone. Here again correlation of blood levels will be made with MFO, chemical body burdens and measures of reproductive success.

References

Buty, S. G., S. Thompson and F. J. Slaga. 1976. The role of epidermal aryl hydrocarbon hydroxylase in the covalent binding of polycyclic hydrocarbon to DNA and its relationship to tumor initiation. Biochem. Biophys. Res. Commun. 70, 1102-1108.

Felton, J. S. and R. L. Dobson. 1983. The mouse oocyte toxicity assay, in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III, pp. 245-255. (Waters, Sandu, Lewtas, Claxton, Chernoff and Nesmow, Eds.) Plenum Publishing, New York, NY.

Felton, J. S., D. S. Orwig, L. E. Olsen, G. S. Knize, G. J. Ryan, T. C. Kwan, and R. L. Dobson. 1980. Oocyte killing in mice as an in vivo gametic cytotoxicity assay: results for 45 chemicals. Environ. Mutag. 2, 213

Hose, J. E., J. B. Hannah, M. L. Landolt, B. S. Miller, S. P. Felton and W. T. Iwaoka. 1981. Uptake of benzo(a)pyrene by gonadal tissue of flatfish (family pleuronectidae) and its effect on subsequent egg development. J. Toxicol. Environ. Health 7, 991-1000.

Kouri, R. E., R. A. Salerno and C. E. Whitmire. 1973. Relationships between aryl hydrocarbon hydroxylase inducibility and sensitivity to chemically induced subcutaneous sarcomas in various strains of mice. J. Natl. Cancer Inst. 50, 363-368.

Phillips, D. H., P. L. Grover and P. Sims. 1978. The covalent binding of polycyclic hydrocarbons in DNA in the skin of mice of different strains. Int. J. Cancer 22, 487-494.

Spies, R. B., J. Felton and L. Dillard. 1982. Hepatic mixed-function oxidases in California flatfishes are increased in contaminated environments and by oil and PCB ingestion. Mar. Biol. 70, 117-127.

Stegeman, J. J. 1980. Mixed-function oxygenase studies in monitoring for effects of organic pollution. Rapp. P. -v. Reun. Cons. perm. int. Explor. Mer. 179, 33-38.

Stegeman, J. J. and M. Chevion. 1979. Sex differences in cytochrome P-450 and mixed function oxygenase activity in gonadally-mature trout. Biochem. Pharmac. 29, 553-558.

Ungvary, G. B. Varga, E. Horvath, E. Tatrai and G. Folly. 1981. Study on the role of maternal sex steroid production and metabolism in the embryo toxicity of para-xylene. Toxicology 19, 263-268.

Varanasi, U., D. J. Gmur and M. M. Krahn. 1980. Metabolism and subsequent binding of benzo(a)pyrene to DNA in pleuronectid and salmonid fish, in Polynuclear Aromatic Hydrocarbons: Fourth International Symposium on Analysis, Chemistry, and Biology, pp. 455-470, Battelle Press, Columbus, Ohio.

Varanasi, U., M. Nishimoto, W. L. Reichert and J. E. Stein. 1982. Metabolism and subsequent covalent binding of benzo(a)pyrene to macromolecules in gonads and liver of ripe English sole. (Parophrys vetulus). Xenobiotica 12, 417-425.

Walton, D. G., W. R. Penrose and J. M. Green. 1978. The petroleum inducible mixed function oxidase of cunner (Tautogolabrus adspersus Walbaum 1792): some characteristics relevant to hydrocarbon monitoring. J. Fish Res. Bd. Can. 35, 1547-1552.

Westernhagen, H. von, H. Rosenthal, V. Dethlefson, W. Ernst, U. Harms and P. D. Hansen. 1981. Bioaccumulating substances and reproductive success in Baltic flounder Platicthys flesus. Aquatic Toxicol. 1, 85-100.

DISCLAIMER

Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161
Price: Printed Copy $; Microfiche $4.50

Page Range	Domestic Price	Page Range	Domestic Price
001-025	$ 7.00	326-350	$ 26.50
026-050	8.50	351-375	28.00
051-075	10.00	376-400	29.50
076-100	11.50	401-426	31.00
101-125	13.00	427-450	32.50
126-150	14.50	451-475	34.00
151-175	16.00	476-500	35.50
176-200	17.50	501-525	37.00
201-225	19.00	526-550	38.50
226-250	20.50	551-575	40.00
251-275	22.00	576-600	41.50
276-300	23.50	601-up[1]	
301-325	25.00		

[1]Add 1.50 for each additional 25 page increment, or portion thereof from 601 pages up.